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LABORATORY EVALUATION OF THE TOXICITY OF NITROGLYCERINE TO AQUATIC ORGANISMS.

by

R.E. Bentley, J.W. Dean, S.J. Ells, G.A. LeBlanc
S. Sauter, K.S. Buxton, and B.H. Sleight, III

FINAL REPORT
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The toxicity of nitroglycerine to a wide variety of aquatic organisms representing several different trophic levels in aquatic ecosystems was studied. The results of static acute toxicity tests indicate that the nominal acute LC50 values are generally greater than 1 mg/l nitroglycerine. A pilot bioaccumulation study with 14C-nitroglycerine showed that there was no appreciable accumulation of 14C-nitroglycerine in the edible tissues of four fish species. The estimated application factor for fathead minnow over one complete life cycle is >0.01<0.02 and >0.03<0.07 for eggs and fry of the fathead minnow and channel catfish, >0.2<0.4 for daphnids

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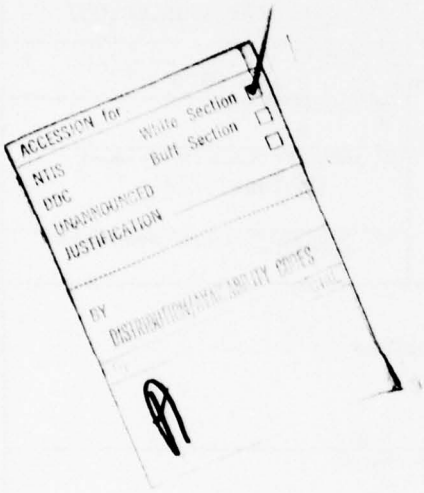
and ~~>0.3<0.6~~ for midges. Applying the lower limit of all estimates of the application factor (0.03) to the lower limit of the observed acute toxicity values for freshwater organisms (1.28 mg/1, 96-hour LC50 for bluegill at pH 6.0), we propose a water quality criterion of 0.01 mg/1 nitroglycerine.



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SUMMARY

In order to provide the data base required to perform a hazard evaluation relative to the occurrence of nitroglycerine in the aquatic environment and to recommend a proposed water quality criterion for nitroglycerine for the protection of freshwater aquatic life, acute, subacute and chronic toxicological evaluations were performed on a wide variety of freshwater organisms representing several trophic forms utilizing nitroglycerine. Additionally, the bioconcentration of 14C-nitroglycerine in fishes was investigated.

The results of static acute toxicity tests indicate that the nominal acute LC50 values generally are greater than 1 mg/l nitroglycerine. Nitroglycerine was generally about 10X less toxic to the four species of invertebrates than to the fishes and two of the phytoplankton species during static acute toxicity tests. In flow-through toxicity tests, nitroglycerine again appeared to be about 10X less toxic to the species of invertebrates than to fishes. Nitroglycerine was not appreciably more toxic to both fishes and invertebrates during flow-through toxicity tests than during static toxicity tests suggesting little cumulative toxicity of nitroglycerine as a result of duration of exposure.

The results of the pilot bioaccumulation study with 14C-nitroglycerine indicate an apparent lack of appreciable accumulation

of 14C-nitroglycerine in the edible tissue of four fish species tested. The estimated bioaccumulation factor for nitroglycerine in fish during this test ranged from 8-15X. A decrease (~50%) in 14C-residues was observed in two of the fish species tested despite continued exposure to 14C-nitroglycerine which suggests that some type of enzyme induction may have occurred.

Based on the results of the chronic toxicity study with fathead minnows, the critical life stage study with fathead minnows, and the chronic studies with daphnids and midges, we estimate that the application factor specific for nitroglycerine which describes the relationship between chronic and acute toxicity is >0.037<0.073 for fathead minnow over one complete life cycle, >0.01<0.02 and >0.03<0.07 for eggs and fry of the fathead minnow and channel catfish, >0.2<0.4 for daphnids, and >0.3<0.6 for midges.

Based on a conservative analysis, we have applied the lower limit of all estimates of the application factor specific for nitroglycerine (0.03) to the lower limit of the observed acute toxicity values for freshwater organisms (1.38 mg/l, 96-hour LC50 for bluegill at pH 6.0). As a result of this analysis, we propose that a concentration of 0.01 mg/l nitroglycerine would be a reasonable water quality criterion providing for the protection of freshwater aquatic life with an ample margin of safety.

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INTRODUCTION

Nitroglycerine is known to occur in discharges from several Army Ammunitions Plants (AAP's). An estimation has been made that concentrations of nitroglycerine in receiving streams adjacent to AAP's would be in low parts per billion range, on the order of 1-10 ug/l (Rosenblatt et al., 1973). Since a review of the scientific literature indicated there is no information on the toxicity of nitroglycerine to aquatic organisms (Dacre and Tew, 1973) a program was undertaken to investigate the acute and chronic toxicity of nitroglycerine to aquatic organisms. The objectives of the program were to provide the data base required to perform a hazard evaluation relative to the occurrence of nitroglycerine in the aquatic environment and to recommend a proposed water quality criteria for nitroglycerine for the protection of freshwater aquatic life with ample margin of safety.

The specific efforts undertaken included investigations of: (a) the acute toxicity of nitroglycerine to a variety of aquatic organisms under static and flowing water (flow-through) conditions; (b) the effects of variations in water quality on the acute toxicity of nitroglycerine to fish; (c) the chronic toxicity of nitroglycerine to both aquatic vertebrate and invertebrate organisms; and (d) the bioconcentration of nitroglycerine by fishes.

The studies to evaluate the acute toxicity of nitroglycerine to phytoplankton were performed at the Marine Research Laboratory of E G & G, Bionomics in Pensacola, Florida. The studies to evaluate the toxicity of nitroglycerine to all other aquatic organisms and the bioconcentration of this chemical by fishes were conducted at the Aquatic Toxicology Laboratory of E G & G, Bionomics in Wareham, Massachusetts.

MATERIALS AND METHODS

Test Material

The nitroglycerine used for these investigations was supplied as a 10% mixture in lactose by both E. I. Dupont de Nemours Co., Wilmington, Delaware and I C I United States, Goldsboro, North Carolina. The nitroglycerine was quantitatively separated from the lactose by mixing with acetone. The lactose is essentially insoluble in acetone and rapidly precipitated, while nitroglycerine is extremely soluble in acetone (Chemical Rubber Co., 1971). Thus, solutions of lactose-free nitroglycerine in acetone could be readily prepared by decanting the supernatant from the nitroglycerine in lactose: acetone mixtures. Concentrations of test materials are reported as milligrams (mg) of active ingredient per liter (l) of diluent water, or parts per million (ppm). For algal assays nitroglycerine in lactose was utilized as the test material and results are expressed as mg nitroglycerine/liter of water (ppm).

The 14C-nitroglycerine used in the bioaccumulation study was received in a vial from Dr. Ching-Chun Lee of the Midwest Research Institute, Kansas City, Missouri which was received by him as uniformly labeled 14C-nitroglycerine from New England Nuclear Corporation, Boston, Massachusetts. Correspondence which accompanied the vial stated that it contained 1.01 millicuries of 14C-nitroglycerine in 21 ml of methanol at a concentration of 10 mg 14C-nitroglycerine/ml of methanol. The entire contents of this vial were transferred to a 100-ml volumetric flask

and brought to volume with acetone and served as a ^{14}C-nitroglycerine solution from which working stock solutions were prepared for this study.

Test Organisms

Algae tested were the cyanophytes (blue-greens) Microcystis aeruginosa and Anabeana flos-aquae; the chlorophyte (green) Selenastrum capricornutum; and the chrysophyte (diatom) Navicula pelliculosa. Cultures were obtained from the collection at the University of Indiana, Bloomington, Indiana, and the Pacific Northwest Water Quality Laboratory (EPA, Corvallis, Oregon). Each species was maintained in stock cultures at Bionomics Marine Research Laboratory. Culture medium was prepared according to the formula described in "Algal Assay Procedure: Bottle Test" (U.S. EPA, 1971a).

Macroinvertebrates exposed to nitroglycerine were the water flea (Daphnia magna), scud (Gammarus fasciatus), sowbug (Asellus militaris), and midge (Chironomus tentans). The D. magna were acquired from Bionomics' laboratory cultures, and the scud, sowbug, and midge were collected in the Wareham, Massachusetts area by Bionomics' personnel.

At the initiation of testing, water flea were 0-24 hours old,

scud and sowbug were in the juvenile stage; and the midge larvae were in the second or third instar stage.

Fish utilized in acute, static bioassays were bluegill (Lepomis macrochirus), rainbow trout (Salmo gairdneri), channel catfish (Ictalurus punctatus) and fathead minnow (Pimephales promelas). Unless otherwise noted, the bluegill were acquired from a commercial fish farmer in Nebraska, and had a mean ($\pm$ S.D.) weight of 1.0 ($\pm$ 0.3) g and a mean ($\pm$ S.D.) standard length of 35 ($\pm$ 6) mm. The rainbow trout were acquired from a commercial trout producer in Massachusetts and had a mean weight and length of 0.9 ($\pm$ 0.3) g and 43 ($\pm$ 4) mm, respectively. The channel catfish were obtained from a fish farmer in Arkansas and had a mean weight of 1.2 ($\pm$ 0.5) g and a mean length of 57 ($\pm$ 11) mm. The fathead minnow were obtained from a commercial producer in Arkansas, and had a mean weight of 1.9 ($\pm$ 0.4) g and a mean length of 43 ($\pm$ 8) mm. For all tests, thirty fish representative of test populations of each species were weighed and measured for the calculation of means and standard deviations for each group.

Flow-through bioassays with nitroglycerine were conducted with bluegill obtained from a commercial hatchery in Connecticut and had a mean weight of 2.0 ($\pm$ 0.2) g and mean standards length of 41 ($\pm$3) mm; fathead minnows acquired from a commercial hatchery in Arkansas and had a mean weight and length of 1.5

(± 0.2) g and 44 (± 4) mm, respectively, channel catfish were acquired from a commercial fish farmer in Missouri and had a mean weight and length of 2.1 (± 0.2) g and 45 (± 3) mm, respectively.

To investigate the bioconcentration of ^{14}C-nitroglycerine by fishes, bluegill, fathead minnows, channel catfish and rainbow trout were obtained from various commercial hatcheries. The mean weight and standard lengths of the fishes utilized for these studies were: bluegill, 2.1 (± 0.3) g and 42 (± 4) mm; fathead minnows 2.3 (± 0.4) g and 59 (± 6) mm; channel catfish 1.4 (± 0.2) g and 46 (± 4) mm; and rainbow trout 1.2 (± 0.3) g and 48 (± 5) mm.

To investigate the chronic toxicity of nitroglycerine to fathead minnows, fry (<24 hours old) were received from the EPA, Environmental Research Laboratory in Newton, Ohio.

Prior to use in tests, all fish were held in 1700-ℓ concrete raceways which were coated with an epoxy resin paint to prevent leaching of materials into the water. Flow of well water (temperature, 20 ± 1.0°C for bluegill, channel catfish, and fathead minnow, and 14 ± 1.0°C for the rainbow trout; hardness, 35 mg/ℓ as $CaCO_3$; pH 7.1, and dissolved oxygen concentration, >60% of saturation) into these raceways was at a minimum rate of 4 l/

minute, which provided an adequate water turnover for holding these species. The fishes were maintained in these laboratory hatchery facilities for at least thirty days prior to use in bioassays. During this period, cumulative mortality for each species was <2%; no mortality was observed during the 48 hours immediately prior to testing, and these fishes were judged to be in excellent condition. Fish of each species were from the same year class, and the standard length of the longest fish was no more than twice that of the shortest fish.

Test Methods

(A) Static Acute Toxicity Tests - In order to evaluate the relative susceptibility of a broad spectrum of aquatic organisms to nitroglycerine, static bioassays were conducted.

During all bioassays to investigate the acute toxicity of nitroglycerine to aquatic organisms, two series of concentrations were established within each bioassay, a series of range-finding concentrations (preliminary test) and a series of definitive concentrations (definitive test). The preliminary test was conducted to determine an approximate range of concentrations for evaluating the dose-response relationship. The definitive test, consisting of at least five concentrations, evaluated the dose-response relationship to a degree allowing the median

effective concentration (EC50) or the median lethal concentration (LC50) to be calculated from the data with optimum accuracy.

Algal assays were conducted according to the method described in "Algal Assay Procedure: Bottle Test" (U.S. EPA, 1971a). During algal bioassays, nitroglycerine in lactose, was added with an acetone solvent/carrier. An equal volume of acetone (0.1 mℓ) was added to each flask, including controls. Because the nitroglycerine was combined with lactose, and acetone was used as a solvent/carrier, preliminary tests were conducted to determine the effect of each chemical on the test algae. Acetone was tested at a concentration of 2000 ppm, representing the greatest amount used in any test, and lactose was tested at 1, 10 and 100 ppm, representing a range of concentrations which could occur in these tests.

To determine the effects of nitroglycerine on algae, measurements were made of the chlorophyll a content of exposed and control cultures of each of the four test species. In addition, to confirm these results, determinations of cell numbers for cultures of M. aeruginosa, S. capricornutum and N. pelliculosa and of optical density for A. flos-aquae were performed.

Chlorophyll a analyses were conducted according to the procedures of Strickland and Parsons (1972) and involved filtering

algal cultures from test medium, extracting chlorophyll by treatment of algal cells with acetone, determining extinction values with a spectrophotometer and finally, calculating the chlorophyll a concentration in the solution. Chlorophyll a and optical density measurements (at 680 nanometers) were made with a Bausch & Lomb Spectronic 20 spectrophotometer. Cell counts were performed with a compound light microscope and a hemacytometer. In each case, the measurements obtained from triplicate exposed cultures were averaged, the results compared with those from triplicated controls and a percentage effect (relative to controls) was calculated.

Each test concentration was converted to its logarithms and the corresponding percentage effect (change in chlorophyll a concentration, optical density or cell number) converted to a probit. The 24-, 48- and 96-hour median effective concentrations, EC50's (concentrations effective in changing the chlorophyll a concentration, optical density or cell number of exposed algae by 50% as compared to controls) and their 95% confidence limits were then estimated from a linear regression equation calculated with a programmable calculator.

Test methods used for static bioassays with macroinvertebrates and fishes were as described in "Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians" (U.S. EPA, 1975).

Results of macroinvertebrate bioassays are expressed as EC50's (concentrations effective in causing immobilization of 50% of test animals) and results of fish bioassays are expressed as LC50's (concentrations lethal to 50% of test animals). The EC50 and LC50 values and their 95% confidence limits were estimated from a linear regression equation calculated with a programmable calculator. Data from replicates were averaged and utilized in the regression analysis.

Macroinvertebrate bioassays were conducted in 250-ml beakers containing 200 ml of solution at 20 $\pm$ 1.0°C. Aged well water (hardness, 35 mg/l as $CaCO_3$; pH, 7.1) was utilized in the performance of these bioassays.

Dissolved oxygen values in test vessels during static bioassays with invertebrates ranged from 8.0 to 8.2 mg/l throughout the testing period. Macroinvertebrates were introduced into test beakers within 30 minutes following addition of the nitroglycerine; 15 animals of each species were tested at each concentration (3 replicates, 5 animals/replicate). Static fish bioassays were conducted in 19.6 liter glass vessels containing 15 liters of test solution held in constant temperature water baths at 20 $\pm$ 1.0°C for bluegill, channel catfish, and fathead minnow, and at 10 $\pm$ 1.0°C for rainbow trout. The standard diluent (well water) for the fish species had a hardness of 35 mg/l as $CaCO_3$ and pH of 7.1. Dissolved

oxygen values in various test vessels during bioassays with fishes ranged from 9.0 initially to 4.0 mg/ℓ at the end of the tests. Fish were introduced into each test vessel within 30 minutes after the compound was added; 30 animals of each species were utilized for each concentration (3 replicates; 10 animals/replicate).

Fathead minnows were chosen as the test species to evaluate the relative susceptibility of life stages of fish to nitroglycerine because of the ability to readily procure their various life stages in the laboratory. The susceptibility of selected life stages (egg, 1-hour old newly-hatched fry, 7-day old fry, 30-day old fry, and 60-day old fry) of fathead minnow (Pimephales promelas) to nitroglycerine was evaluated under static bioassay conditions for a 144-hour period with the eggs, and for a 96-hour period with all other life stages. The egg, 1-hour old fry and 7-day old fry bioassays were conducted in 250-mℓ beakers containing 200 mℓ of solution (10 animals/beaker, 3 replicates/concentration, 30 animals/concentration). The 30-day old fry and 60-day old fry bioassays were conducted in 1-gallon glass jars containing 3 ℓ of solution (10 fry/jar, 3 replicates/concentration, 30 animals/concentration). The LC50 values for the egg tests were calculated at 24, 48 and 144 hours. The time period of 144 hours allowed 100% hatch of eggs in all control beakers. In addition to percent mortalities,

percent hatch of eggs was also observed. These tests were conducted at 25 $\pm$ 1.0^{o}C, and the standard diluent had a pH of 7.1 and total hardness (EDTA) of 35 mg/ℓ as $CaCO_3$.

Due to their sensitivity to the chemicals, their availability, and their expected presence in most of those areas where nitroglycerine might be found, bluegill were selected as the test species to evaluate the effect of water quality on the toxicity of nitroglycerine. The susceptibility of bluegill to nitroglycerine under various water quality conditions was evaluated during static bioassays for a 96-hour period. The bluegill used in these tests were obtained from a commercial fish farmer in Nebraska and had a mean ($\pm$ S.D.) wet weight and standard length of 0.9 ($\pm$ 0.2) g and 33 ($\pm$ 5) mm, respectively. Bioassays were conducted utilizing bluegill to determine the 24, 48 and 96 hour LC50 values of nitroglycerine: a) at three temperatures representing the lower end (15^{o}C), mid-point (20^{o}C), and upper end (25^{o}C) of the normal temperature range for bluegill using soft water (35 mg/ℓ $CaCO_3$) at neutral pH; b) in soft water (35 mg/ℓ $CaCO_3$), in hard water (100 mg/ℓ $CaCO_3$) and in very hard water (250 mg/ℓ $CaCO_3$) using water of pH 7.0 at the recommended test temperature of 20^{o}C; and c) at pH's of 6.0, 7.0 and 8.0 using standard soft water at the recommended test temperature of 20^{o}C. The diluent for each of these conditions was prepared according to the procedures recommended by Marking and

Dawson (1973). Dissolved oxygen values in various test vessels during these bioassays ranged from 9.0 initially to 4.2 at the end of the tests.

General availability and their expected presence in those areas where the nitroglycerine might be found resulted in the use of bluegill to evaluate the stability of the toxicological properties of dissolved nitroglycerine. The susceptibility (LC50) of bluegill (Lepomis macrochirus) to nitroglycerine was evaluated under static bioassay conditions for a 96-hour period utilizing solutions which were "aged" for 0,12,24,48 and 96 hours prior to initiating the toxicity tests. Fish (10 fish/replicate, 3 replicates/concentration) were introduced into aged test solution at each time period. The bluegill used in these tests were acquired from a commercial fish farmer in Nebraska, and had a mean ($\pm$ S.D.) wet weight of 0.8 ($\pm$ 0.2) g and a mean ($\pm$ S.D.) standard length of 32 ($\pm$ 4) mm. The standard diluent had a pH of 7.1 and a total hardness (EDTA) of 35 mg/ℓ as $CaCO_3$.

In order to investigate the possible degradation of nitroglycerine under various test conditions, one concentration of nitroglycerine was maintained (without fish) under each set of water quality conditions. Water samples were removed from these test vessels at 0, 24, 48 and 96 hours.

(B) Flow Through Acute Toxicity Tests - Procedures used in these toxicity tests were based on protocols described in "Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians" (EPA, 1975) except where stated otherwise.

The flow-through bioassays were conducted using an intermittent-flow proportional dilution apparatus (Mount and Brungs, 1967). The apparatus provides for intermittent introduction of seven (fish) and six (invertebrates) concentrations of the test compound into 30-liter (fish) or 1.75 ℓ (invertebrates) and diluent water to a vessel serving as a control unit. The control vessel received solvent (acetone) at a concentration equivalent to the greatest amount of acetone introduced to any test vessel. During bioassays with fishes, the flow rate of test solutions to each 30-ℓ aquarium was 5 ℓ/hour throughout the test period. During the flow-through invertebrate bioassays, flow rate to each 1.75-ℓ aquarium was 4.0 ℓ/day. Thirty fish were randomly assigned to each test vessel within 30 minutes after the compound was added. Twenty daphnids or chironomids were randomly assigned to each replicate test chamber within 30 minutes after the nitroglycerine was added.

Diluent water used in these tests had the same water quality characteristics as previously described for holding water. All test vessels were maintained in water baths at 21 $\pm$ 1.0°C and

test solutions were not aerated during the test. During these tests, the dissolved oxygen concentration, pH and temperature of test solutions were checked at various intervals during exposure in the highest, middle and lowest test concentrations, at a minimum. DO and temperature were measured with a YSI dissolved oxygen meter and combination oxygen-temperature probe; pH was measured with a Corning Digital pH meter and probe.

Results of the definitive tests were expressed as the time-dependent (24- and 96-hour) LC50, and time-independent (incipient) LC50, the nominal concentration of test compound in diluent water which caused 50% mortality in test populations of fish and invertebrates with no additional significant response (<10%) during the final 48 hours of exposure. Test concentrations and corresponding observed percentage mortalities were converted to logarithms and probits, respectively, and these values were utilized in a least squares regression analysis. The LC50's and their 95% confidence intervals were calculated from the regression equation.

(C) Pilot Bioaccumulation Study of Nitroglycerine In Fishes - In order to determine the bioaccumulation potential of nitroglycerine to various fish species in water, a pilot bioaccumulation study was conducted for 8 days. A modified intermittent-

flow, dilution apparatus (Mount and Brungs, 1967) was used to deliver a nominal concentration of 0.50 mg/l 14C-nitroglycerine in Bionomics' well water, and control water (well water) to duplicate 30-liter glass aquaria at a rate of five liters/hour.

The 14C-nitroglycerine stock solution to be used in this study was prepared by placing 5.0 ml of the 14C-nitroglycerine solution (10.5 mg nitroglycerine) and 7.2 ml of the unlabelled nitroglycerine 348.7 mg nitroglycerine) in a 100 ml volumetric flask and bringing to volume with acetone. This yielded a superstock concentration of 3.7 mg 14C-nitroglycerine/ml acetone with a theoretical specific activity of 308 disintegrations per minute/ug nitroglycerine. To determine the measured specific activity of this superstock, three 0.2 ml aliquots of superstock were transferred to glass scintillation vials containing 15 ml of counting solution (Monophase, Packard Instrument Co.) and the vials were placed in a liquid scintillation spectrometer and the activity counted. The mean measured specific activity of the 14C-nitroglycerine in this superstock was determinated to be 317 $\pm$ 24 dpm/ug, 103% of the theoretical activity.

A container to promote mixing of the 14C-nitroglycerine diluter stock and diluent water was utilized prior to delivery of the test solution to the exposure aquarium.

On April 21, 1976, 20 each of bluegill, fathead minnows, channel catfish and rainbow trout were placed in each of two aquaria to initiate the exposure period. Fish in both aquaria were fed Agway "Strike" dry pelleted feed ad libitum daily and fecal material was siphoned every other day. The water temperature in each aquarium was maintained at 18 ± 1.0°C. In order to maintain a dissolved oxygen concentration >60% of saturation, the test water was continuously aerated.

Water and fish samples were taken from the exposure aquarium on days 1, 2, 4 and 8 and from the control aquarium on day 8. On each sampling day, triplicate 5.0 mℓ water samples were pipetted directly into scintillation vials containing 15 mℓ of counting solution. The vials were then placed in a Model 2112 Packard Tri-Carb Liquid Scintillation Spectrometer for quantitation of ^{14}C-nitroglycerine.

Four fish of each species were withdrawn on each sampling day and filleted. Duplicate samples of the edible tissue (fillet) were wet weighed and air-dried for ca 24 hours in combustion cones at 21°C. Each dried sample was then combusted in a Packard Model 306 Sample Oxidizer. The resulting $^{14}CO_2$ was trapped as a carbonate in a mixture of Carbosorb (1M hyamine hydroxide in methanol) and scintillator fluid (4 g 98% PPO + 2% MSB/liter toluene) into a scintillation vial. The vials

were then placed in the spectrometer and the activity of each sample was determined.

Recovery rates of the oxidizer were determined prior to analyzing each set of samples by combusting and counting the activity of a standard reference material (New England Nuclear Corp.) and comparing the measured value to the known theoretical value of the standard. The counting efficiency (E) for each sample was determined as follows. Aliquots of a ^{14}C-toluene standard, obtained from New England Nuclear with an accurately known specific activity were added to a series of counting vials containing counting solution of a type and volume identical to those used for counting water or combusted tissue samples. Increasing volumes of nitromethane, a chemical quenching agent, were then added to individual vials in each series. The vials were counted with two channels simultaneously and the channel ratios determined. The counting efficiency of each standard was determined as the quotient of counts per minute divided by the disintegrations per minute of the standard. From these data, a graph of counting efficiency versus channel ratio was constructed for each of the two types of samples (water and tissue). Counting efficiences of all test samples were determined by calculating the channel ratio and interpolating the corresponding counting efficiency from the graph (Bransome, 1970). By this method, counting efficiencies for ^{14}C-nitro-

glycerine ranged from 78%-79% for water and from 51%-68% for fish tissue.

Background levels of radiation for water and fish were determined by analyzing control samples prior to the initiation of the study and were measured to be 28 cpm for water and fish. All samples were counted for a minimum of 100 minutes or until 5,000 counts were generated. Using this procedure and the calculations described in Standard Methods for the Examination of Water and Wastewater (APHA, 1971) it was determined (at the 95% confidence level) that the minimum detectable activity above mean background levels (minimum net cpm) was 22 cpm. The lowest net cpm of any tissue sample measured during the study was 27. A maximum 6.6% counting error is associated with this value. This is the maximum counting error, as the percentage counting error for each sample depends on the net cpm of that sample and varies inversely with the activity. The minimum detectable ^{14}C-residue concentration for edible tissue is a range as it depends on the efficiency and weight of each sample. The end points of the range are calculated by using the lowest efficiency and lightest sample weight measured (maximum limit) and the highest efficiency and the heaviest sample weight (minimum limit).

The calculations used in determining the concentration of ^{14}C-nitroglycerine or ^{14}C-residues in each sample were the following:

i. net counts/minute (cpm) = (sample-background) cpm

ii. counting efficiency (E) = from channel ratio method

iii. disintegrations per minute (dpm) in counted sample = $\frac{\text{net cpm}}{\text{counting efficiency (E)}}$

iv. total ^{14}C-residues calculated as nitroglycerine (µg/ℓ) (µg/mℓ or µg/g) = $\frac{\text{dpm in combusted sample}}{\text{specified activity of the nitroglycerine superstock x sample size (dpm/µg) x g or mℓ)}}$

Sample Calculation

$$\frac{\text{net cpm (619)/E (0.57)}}{\text{specific activity x sample wt.}} = \frac{\text{net cpm (619)/E (0.57)}}{\frac{317}{\text{(0.616 g)}}}$$

= 5.6 mg/kg ^{14}C-residues calculated as nitroglycerine

Each set of daily sample measurements was examined for "outliers", or extreme observations, according to the method of Dixon and Massey (1957). Any values determined not to be from the same "population" as the other values in that set (P=0.05) were discarded. A mean nitroglycerine concentration was then calculated from the measured activity of the samples determined for each sampling day.

(D) Critical Life Stage Studies - The effects of 30 days continuous exposure to nitroglycerine on the eggs and fry of

channel catfish and fathead minnows were investigated using the procedures recommended by U.S. EPA (1972). In addition, due to the high mortality observed during the first fathead minnow study, a second study was initiated with fathead minnow. This study was run for 7 days, at which point it was terminated due to the verification of the previous data, and reset at lower concentrations in a third study. A proportional diluter (Mount and Brungs, 1967) modified with a McAllister (1972) chemical metering device delivered water and toxicant at a diluter factor of 0.5 to the test aquaria. Chemical stock solutions were dissolved in nanograde acetone and distilled water to a volume of two liters. Stock solutions were shielded from light throughout the test. A magnetic stirring device was placed under each stock solution and moderate stirring was maintained in the stock solution throughout the test. For the second and third fathead minnow studies, the stock solution was delivered to the mixing chamber by means of a 50 ml glass syringe with a stainless steel needle. Stock solutions were prepared weekly. Five concentrations, a control and solvent control flowed through separate glass delivery tubes to duplicate test aquaria. Thus, each concentration and each control was replicated and each replicate was designated as either A replicate or B replicate. Test aquaria measured 30 x 35 x 30 cm. Each test aquarium contained two growth chambers (13 x 30 x 27 cm) designated A and B, which had stainless steel 40 mesh screen affixed to one end allowing water to drain out while retaining the young fish. A constant-level drain tube

extending 15 cm above the bottom of each test aquarium retained a total volume of 15.75 l. Each growth chamber contained a water volume of 5.9 l. A glass, flow-splitting chamber was calibrated to deliver an equal volume to each of the growth chambers. The test water was delivered to all chambers at a mean flow rate of 8.1 tank volumes for channel catfish and 7.4 tank volumes for the first fathead minnow exposure and 6.8 tank volumes for the second and third fathead minnow exposures per 24 hours.

A constant temperature of 22 $\pm$ 1.0^{o}C was maintained for test organisms by placing aquaria in water baths in which circulating water was heated by immersion coil heaters and regulated by mercury column thermoregulators.

Exposure of channel catfish eggs began within 48 hours after fertilization. Eggs were obtained from a catfish farmer in Missouri. Fifty eggs were randomly distributed to each egg cup. Eggs were treated each day during the first three days of exposure with a 3 minute dip in a 30 ppm solution of malachite green to retard fungus. Dead eggs were removed and counted each day until hatching was completed (8-9 days after exposure began). Subsequently, 25 fry were randomly selected and transferred to the larval growth chambers. Fry were fed brine shrimp nauplii ad libitum twice per day and Agway Strike Trout Starter once per day, beginning 5 days after hatching was completed and continuing throughout the larval exposure period.

Exposure of fathead minnow eggs began within 24 hours after fertilization. Eggs were obtained from brood stocks at the Aquatic Toxicology Laboraotry of E G & G, Bionomics in Wareham, Massachusetts.

At least thirty five eggs were randomly distributed to each egg cup. Dead eggs were removed and counted each day until hatching was completed (3-5 days after exposure began at 22°C). After hatching was completed, 25 fry were randomly selected and transferred to the larval growth chambers. Fry were fed brine shrimp nauplii ad libitum twice per week, beginning one day after hatching was completed and continuing throughout the exposure period.

For both species the two egg cups were oscillated in their respective test vessels by means of an egg cup rocker arm apparatus (Mount, 1968). Percentage hatch was based on the number of live fry in the egg cup when hatching was completed. After fry were transferred, growth chambers were siphoned twice weekly to remove fecal material and excess food. With the exception of the second fathead minnow test, total length of each group of fry was determined at 30 days post-hatch using the photographic method of McKim and Benoit (1971). Percentage survival at 30 days post-hatch was also recorded at this time.

Based on acute toxicity information, nominal concentrations to be studied during embryo-larval studies ranged from 0.07-1.25 mg/l for channel catfish, and ranged from 0.03-3.75 during the various fathead minnow studies.

At the initiation of the channel catfish test, the highest concentration of solvent in the test chamber was 116 mg/l. Problems with nitroglycerine coming out of solution and forming globules in the chemical stock bottle necessitated raising the percentage solvent (acetone) in the stock solution. Beginning on test day 13 post-hatch, the highest concentration of acetone in a test chamber was 155 mg/l. However, the solubility problem still persisted throughout the test with small globules of nitroglycerine forming in the stock bottles. The concentration of acetone in the solvent control was 125 mg/l from day 1-13 post-hatch, and 155 mg/l from day 13-30 post-hatch.

Solubility problems were also encountered during the first fathead minnow study which was performed concurrently. On day 0 of this study, the highest concentration of solvent in a test chamber was 116 mg/l, on day 4 this concentration was increased to 193 mg/l, on day 14, to 290 mg/l and on day 24, to 387 mg/l (100% acetone in stock solution). The concentration of acetone in the solvent control was 125 mg/l on day 0 and was increased proportionally each time the concentration of acetone in the stock solution was raised. During the second and third fathead minnow embryo-larval studies, due to a change in the stock delivery device, the maximum concentration of solvent was reduced to 18 and 9 ug/l, respectively.

The means of the measured biological parameters (from duplicate aquaria) were subjected to analysis of variance according to Steel and Torrie (1960). The data for percentage survival and

percentage hatch were transformed to arcsin $\sqrt{\text{percentage}}$ prior to analysis. When treatment effects were indicated, the means of these effects were subjected to Dunnetts procedure for comparing treatment means with control (Steel and Torrie, 1960). All differences were considered statistically significant at a probability of P=0.05.

(E) Invertebrate Chronic Tests - To investigate the chronic toxicity of nitroglycerine to both midge larvae and daphnids, a proportional dilutor (Mount and Brungs, 1967) with a dilution factor of 0.5 and a syringe injector, delivered the test water and test compound to the mixing chamber, and mixing cells. From the mixing cells, the nitroglycerine solutions were delivered to each of 4 replicate aquaria through individual glass delivery tubes. Nitroglycerine stocks were prepared in 100% acetone. Two of the 4 control replicate aquaria received doses of acetone identical to the greatest amount of acetone delivered to the nitroglycerine treated vessels, to detect any possible acetone related effects. The concentration of acetone in these aquaria was 92 mg/ℓ.

Each experimental unit consisted of cylindrical glass battery jars 18 cm high and 13.5 wide. A 3 x 8 cm notch was cut into the top edge of the aquaria and covered with nytex 40 mesh screen to provide drainage. Cylindrical cages constructed of aluminum 16 mesh screen were affixed to the battery jars to allow for adequate emergence areas for the C. tentans chronic exposure.

The water depth within the aquaria was 15 cm and the volume was 1.75 ℓ. Fifty mℓ of the water was delivered to the individual test vessels every 25 minutes during the water flea exposure. Fifty mℓ of the water were delivered to the individual test aquaria every 8 minutes during the midge larvae exposure. For both species, the initial 3 days of the exposures were maintained under static conditions to prevent damage of the early instar organisms due to turbulence.

Dissolved oxygen and temperature were measured throughout these exposures with a YSI dissolved oxygen meter equipped with a combination oxygen-temperature probe.

Daphnia magna (<24 hours old), procurred from laboratory stock cultures, were used to initiate the chronic exposure. Twenty water flea were randomly assigned to each test aquarium. Observations of survival and production of young were made weekly for each test aquarium. If young were present, they were counted and discarded. On day 21, twenty young from each aquarium were retained for the initiation of the second generation exposure.

Chironomus tentans (<48 hours old), procured from laboratory cultures were used to initiate this chronic exposure. At least fifty organisms were placed in each test vessel. Prior to

the introduction of the test organisms, the aquaria were supplied with a substrate consisting of homogenized paper towel, in water, approximately 1 cm deep, and one hundred 2 and 3 mm diameter sections of glass tubing, 2 to 3 cm long. Early instar midge larvae utilize the paper towel substrate for construction of dwelling tubes, while late instar larvae, generally utilize the glass tubing, where their numbers can be visibly quantitated.

Determination of the survival of larvae was made after the control animals had entered their 4th instar stage. Beginning with the onset of emergence, daily records were kept of emergence, adult survival, pupae survival, and egg production. The data were compiled up to the day at which adult mortality of the control animals was greater than emergence of these controls. At this point the aquaria were cleaned, new substrate was supplied, and the second generation was initiated with larvae originating from egg masses taken from the same treatment level into which they were placed.

The food supplied in these bioassays consisted of homogenized Agway "Strike" and cerophyll (20:1 ratio). The combination was blended in water and was filtered through a stainless steel 102 mesh screen for removal of large particles prior to use. A 0.2 mℓ aliquot of the supernatant of the food solution (35

mg/ml) was pipetted into each aquarium of the water flea test three times daily. Aliquots ranging from 0.2 to 0.4 ml of this solution, depending upon the organic enrichment of the water, were pipetted into each aquarium during the midge larvae chronic exposure three times daily.

Means of the measured biological parameters from duplicate aquaria were subjected to analysis of variance (completely randomized block design, P=0.05). The data for percentage survival and percentage hatch were transformed to arcsin $\sqrt{\text{percentage}}$ prior to analysis. When treatment effects were indicated these means were subjected to Dunnett's procedure (Steel and Torrie, 1960). When a treatment mean was significantly different (P=0.05) from the pooled control mean, that treatment level was considered a toxic effect level.

(F) Fathead Minnow Chronic Study - To investigate the chronic toxicity of nitroglycerine to fathead minnows we closely followed the recommended bioassay procedures for fathead minnow chronic tests issued by the Environmental Research Laboratory, Duluth, Minnesota (U.S. EPA, 1971b).

A proportional diluter (Mount and Brungs, 1967) with a dilution factor of 0.5 was used to deliver five concentrations of nitroglycerine and two controls to duplicate test aquaria. One control received only the diluent water (negative control) and a second control received a volume of acetone (positive or

solvent control) equal to that which was added to the aquaria containing the highest concentration of nitroglycerine and acetone to which fish were exposed. A stock solution of nitroglycerine, dissolved in acetone was delivered to the mixing container from a 50 ml glass syringe with a stainless steel needle. The amount of acetone added to the highest concentration of nitroglycerine and to the solvent control was 5.4 mg/l. This is less than 0.001 of the 7-day LC50 of acetone to fathead minnows as reported by Cardwell (1975). A flow splitting chamber (Benoit and Puglisi, 1973) was used to promote mixing of the nitroglycerine and diluent water prior to delivering test water to the aquaria through glass tubing. Each glass aquarium (90 x 30 x 30 cm) was subdivided by a stainless steel 40 mesh screen to provide space for two fry chambers (30 x 12 x 35 cm) and a spawning chamber. Each aquarium was duplicated, resulting in two aquaria, four fry chambers and two spawning chambers for each test concentration. The water level in each aquarium was maintained at 15 cm by a standpipe. The flow rate to the duplicate spawning and quadruplicate fry chambers was seven times their volume per 24 hours.

Five spawning tiles made from halved, 7.5 cm-wide sections of 10 cm diameter cement-asbestos drain tile were placed in each spawning chamber with the concave surface downward. Egg groups were incubated in "egg cups" made from 5 cm diameter glass jars with Nytex 40 mesh screen bottoms. The egg cups were oscillated in the test water by means of a rocker-arm apparatus driven by

a 2-rpm motor (Mount, 1968).

A constant temperature of 25 $\pm$ 1.0^{o}C was maintained in the aquaria by placing them in water baths within which circulating water was heated by immersion coils and regulated by a mercury column thermoregulator.

The photoperiod followed the normal daylight hours of Evansville, Indiana (U.S. EPA, 1971b) and was adjusted on the first and fifteenth day of each month beginning with the Evansville daylength of December 1st on the first day of the test as suggested in the protocol. Illumination was provided by a combination of Durotest (Optima F.S.) and wide spectrum Grow Lux fluorescent lights located centrally, 64 cm above the surface of the water in the aquaria. The entire experimental unit was screened with black, polyethylene curtains to prevent disturbance of the fish and to minimize the effect of ambient laboratory lighting on the intended photoperiod.

Temperature and dissolved oxygen concentrations were measured daily, using a YSI dissolved oxygen meter with a combination electrode polarographic probe, in test aquaria on a rotating basis so that all aquaria were checked at least once each week. Total hardness, alkalinity, pH and acidity were measured in each concentration during the test, according to methods described in APHA (1971). Water samples were removed weekly from each aquarium during the first four weeks and every other week for the remainder of the test to monitor nitroglycerine

concentrations.

Due to problems in availability of eggs, the chronic exposure of fathead minnows to nitroglycerine began on August 5, 1975 with fry obtained from the Environmental Research Laboratory, Newton, Ohio (EPA). Twenty fry (<24 hours) were placed in each fry chamber and were fed brine shrimp (Artemia salinia) nauplii ad libitum three times daily for the first 30 days. During the next 30 days, fry were fed frozen brine shrimp three times daily. At 30 and 60 days, percentage survival and mean total lengths were determined using a photographic method (McKim and Benoit, 1971). At 60 days post-hatch, fish from the two fry chambers in each duplicate aquarium were combined and 15 fish were impartially selected and placed in the respective spawning chambers. The remaining fish were wet weighed in groups and frozen for possible tissue analysis of nitroglycerine. While in the spawning chambers, fish were fed frozen brine shrimp twice daily supplemented by Daphnia magna and Agway "Strike" until the study was completed. All tanks were siphoned weekly to remove fecal material and other particulates. Tanks were brushed every other month when algal growth was excessive.

By test day 174, most fish had well developed secondary sexual characteristics and the sex ratio in each aquarium was reduced by selecting three males and six females for continued exposure and discarding the remaining fish. Research at this laboratory has shown that a reduction of the total number of fish and of

the ratio of males to females in the spawning chamber can result in an increased number of spawns and number of viable eggs.

When spawning began (day 185), eggs were removed from the underside of spawning tiles after 1:00 p.m. each day and counted. Fifty eggs from the first ten spawns in each aquarium were placed in individual egg cups suspended in the corresponding test water. Groups of control eggs were transferred to nitroglycerine treated aquaria in which little or no spawning had occurred. Percentage hatch was calculated for each group of eggs incubated and the mean number of spawns per female, eggs per spawn and eggs per female were determined for each duplicate spawning chamber.

Twenty fry from the first two incubated spawns in each aquarium were placed in the corresponding fry chambers and fed brine shrimp nauplii ad libitum three times daily for 30 days. Control fry were transferred to treatment levels where no spawning or poor hatching had occurred. After 30 days exposure, percentage survival and total length of fry were determined photographically and each fry group was weighed to determine average wet weight of surviving fish.

The test was terminated on day 266, after spawning had virtually ceased for a period of one week in all aquaria. Total length, wet weight, sex and gonad condition were determined for each fish and all fish were frozen for possible tissue analysis.

Means of the measured biological parameters from duplicate aquaria were subjected to analysis of variance (completely randomized block design P=0.05). The data for percentage survival and percentage hatch were transformed to arcsin$\sqrt{\text{percentage}}$ prior to analysis. When treatment effects were indicated, these means were subjected to Dunnett's procedure or, where there were insufficient error degrees of freedom (<5) to obtain a d' using Dunnett's test, the test for least significant difference (Steel and Torrie, 1960). When a treatment mean was significantly different (P=0.05) from the control mean, that treatment level was considered a toxic effect level.

Chemical Methods

During the static bioassays to investigate the possible degradation of nitroglycerine in water, 10 ml samples of test solution were removed for analysis. The nitroglycerine was extracted from water by adding 4 g of NaCl to the 10 ml sample in a glass stoppered vessel, adding 1.0 ml of benzene, and shaking the mixture for one minute. After phase separation, an aliquot of the benzene layer was analyzed by gas chromatography.

During all acute and chronic flow-through experiments, 500 ml water samples were taken periodically for gas chromatographic analysis to quantitate mean measured nitroglycerine concentrations. Samples were extracted with three 50 ml volumes of ethyl ether, the extracts were combined, and refrigerated in 200 ml amber glass vessels with Teflon^{R}-lined caps.

The analysis of water samples for nitroglycerine was performed according to methods described by Williams and Murray (1966)

using the following operating conditions:

Instrument: Tracor Model MT-550 with Ni^{63} electron capture detector.

Column: 6' x 4 mm glass column containing 5% DC-200 on silanized 80/100 mesh Supelcoport packing.

Carrier gas: Column, 65 cc N_2/minute at 14 psi back-pressure detector, 30 cc N_2/minute purge.

Temperatures: Inlet: 200°C Outlet: 210°C

Column: 125°C Detector: 300°C

Recorder: Corning Model 841, 0-1 mv full scale, 0.5/minute chart speed.

Response: 12 ng of nitroglycerine eluted in 4.2 minutes and gave half-scale recorder response at an electrometer attenuation of 1.6 c 10^{-9} amperes.

Seven quality control samples containing 1.0 and 10 mg nitroglycerine per liter were analyzed with 77 $\pm$ 7.5 and 83 $\pm$ 6.7 percent recovery, respectively.

RESULTS

(A) Static Acute Toxicity Tests - Nitroclycerine was generally about 10 X less toxic to the invertebrates than to the fishes and two of the phytoplankton species (M. aeruginosa and A. flos-aquae) during static acute toxicity tests. The acute toxicity of nitroglycerine to all of the invertebrates tested was remarkably similar; the 48-hour EC50 values ranged from 46-55 mg/l. The acute toxicity to fishes was remarkably similar; the 96-hour LC50 values ranged from 2.5-3.2 mg/l. The acute toxicity (96-hour EC50) of nitroglycerine to the phytoplankton species varied over the range of 0.4 mg/l to >10 mg/l depending upon the species tested.

During the phytoplankton bioassays, since any lactose not removed during the dissolution of nitroglycerine in acetone could be a nutrient source for algae, both solvent control and lactose controls were conducted. Test cultures treated with acetone exhibited no differences either in number of cells/ml or chlorophyll a concentrations compared with untreated controls. Exposure to 100 mg/l lactose (equivalent to 10 mg/l nitroglycerine in lactose) had no effect on either the number of cells/ml or chlorophyll a of M. aeruginosa, A. flos-aquae or N. pelliculosa. Exposure to 10 mg/l lactose did result in a 24% increase in the number of S. capricornutum cells/ml compared to untreated controls, and data are corrected for this effect. The

EC50 values for nitroglycerine based on any of the parameters ranged from 0.4 mg/l to >10 mg/l (Table 1). Nitroglycerine depressed the growth of all algae tested except A. flos-aquae as measured by number of cells or optical density (Table 2) or chlorophyll a concentrations (Table 3). The chlorophyte, S. capricornutum was the most sensitive alga tested (96-hour EC50, 0.4 mg/l; based on decrease in number of cells/ml) while the blue green algae were least susceptible to nitroglycerine, with A. flos-aquae being completely unaffected by exposure to 10 mg/l nitroglycerine.

The estimated LC50 values for all fish species and EC50 values for all invertebrate species are summarized (Table 4). No mortality was observed among the fish and invertebrate species exposed to the lactose or acetone controls.

The acute toxicitv of nitroglycerine to various life stages of the fathead minnow varied only slightly (Table 5). The eggs at the time of hatching appeared to be the most sensitive life stafe to nitroglycerine exhibiting a 144-hour LC50 of 1.2 mg/l. Conversely, the 1-hour old fry appeared more tolerant than the older life stages tested.

The acute toxicity of nitroglycerine to bluegill was essentially unaffected by variations in water quality (Table 6). The toxicity appeared to be slightly less at the lowest temperatures tested. Macek et al. (1969) observed similar phenomenon with

the toxicity of many pesticides chemicals to bluegill and trout.

The effect of aging nitroglycerine solutions for periods ranging from 24 to 96 hours had no effect on the toxicity of nitroglycerine to bluegill (Table 7). In fact, the essentially identical 96-hour LC50 values and confidence intervals derived from four of the five sets of solutions tested clearly indicate that nitroglycerine in water is stable for the duration of the standard bioassay period (96-hours). This conclusion is further supported by the results of the gas chromatographic analysis of water samples taken at various time intervals during static bioassays (Table 8). Although, as described in the following section, the absolute accuracy of these data is suspect, the relative stability of the compound in water is clearly evident.

(B) Chemical Analysis Of Nitroglycerine in water - Water samples from the various acute toxicity tests were analyzed according to the methods previously described. Instrument response to successive identical injections of nitroglycerine standards (8 ng) was extremely variable ($\pm$ 50%). Williams and Murray (1966) have suggested that column conditioning (i.e., hourly injections of a large weight of nitroglycerine) is necessary to keep the GC column saturated and achieve reproducible results. We completed the analysis of the above samples (Table 8) and are confident of the relative accuracy of the

measured concentrations but question the absolute accuracy of this method.

Subsequent efforts have been made to utilize methods reported by Reseel and Bogaert (1972) for the gas chromatographic analysis of nitroglycerine. That methodology has similarly been found to be inadequate and unreliable. Most recently, Chandler et al. (1974) have stated "Although GC techniques have apparently been successfully used for the determinations of nitroglycerine at high concentrations, the potential for its decomposition during the course of analysis make the method somewhat questionable for the determinations of trace quantities." These authors recommended a liquid chromatographic method as an alternate approach.

According to the available literature, the concentrations of nitroglycerine tested in this research effort (i.e., 1-10 mg/l) are several orders of magnitude below the reported water solubility of nitroglycerine (1800 mg/l). In addition, we have documented the stability of nitroglycerine in water under a variety of water quality conditions for ± 48 hours. The remaining research efforts were conducted in flow-through systems where the toxicant is effectively introduced on a continuous basis, and radiometric analysis of water samples from the bioconcentration study (cf. part D of this section) indicated that little variance between measured and nominal concentrations occurs. Furthermore, results of analyses of water samples from the third embryo-larval study

with fathead minnows, which were performed by the U.S. Army, confirmed that mean measured concentrations were essentially identical to nominal concentrations. We have, therefore, concluded that the use of nominal concentrations of nitroglycerine as a measure of actual concentrations to which aquatic organisms were exposed is reasonable and accurate.

(C) Flow-through Acute Toxicity Tests - Dissolved oxygen concentrations during flow-through acute toxicity tests with both fishes and aquatic invertebrates were >60% saturation. Nitroglycerine was only slightly more toxic (incipient LC50) to both fishes and invertebrates during flow-through toxicity tests than during static tests (Table 9). Since nitroglycerine has been shown to be relatively stable during static tests, this observation suggests there is little cumulative toxicity of nitroglycerine as a result of duration of exposure, at least in the short term. The fishes (bluegill, fathead minnow and channel catfish) again were approximately 10X more susceptible to nitroglycerine than the invertebrates (midge and water flea). Bluegill and channel catfish were the most sensitive species tested with incipient LC50 values of 0.55 an 0.50 mg/l, respectively.

(D) Pilot Bioaccumulation Study of Nitroglycerine in Fishes - During the eight-day continuous exposure of bluegill, catfish, minnows, and trout to a nominal concentration of 0.50 mg/l 14C-nitroglycerine, no mortalities among any of the species was observed, all fishes appeared normal, fed readily and were considered to be in good physical condition. The mean measured

concentration of 14C-nitroglycerine in the water during the eight-day exposure period was 0.42 ± 0.10 mg/l, equivalent to 85 ± 20% of the nominal concentration (Table 10). Considering the fact that there was a relatively large biomass in the experimental unit which was bioaccumulating the 14C-nitroglycerine to some extent. the slight variance from nominal is expected.

The concentrations of 14C-residues in the edible portion of fishes increased in all four species during the first 4 days of exposure at which time all four species contained 3-6 mg/kg 14C-residues calculated as nitroglycerine (Table 10). Thereafter, no appreciable increase in 14C-residues was observed in bluegill and trout despite an additional 4-day exposure. Furthermore, the 14C-residue concentration present in the edible portion of fathead minnow and channel catfish after 4 days exposure decreased approximately 50% during an additional 4 days of continuous exposure.

Based on a mean measured 14C-nitroglycerine concentration of 0.42 mg/l, during the 8-day exposure, and the mean measured 14C-residue concentration in bluegill (6.2 mg/kg) and trout (3.2 mg/kg) at the end of the 8-day exposure, we estimate the equilibrium bioaccumulation factor for nitroglycerine and the two fish to be ca 15X and 8X, respectively. Based on a mean measured concentration of 14C-nitroglycerine of 0.42 mg/l, and the maximum mean 14C-residue concentration in the edible portion of catfish (3.0 mg/kg) and fathead minnow (3.2 mg/kg), we estimate a maximum bioaccumulation factor for nitroglycerine and these two fishes to be ca 8X.

Based on these data it is apparent that freshwater fishes do not bioaccumulate nitroglycerine from water more than 8-15X. These estimated bioaccumulation factors for nitroglycerine are relatively low when compared to most other chemicals similarly studied. A summary of the results of over fifty bioconcentration tests with bluegill was reported by Macek et al. (1975). These authors suggested that of the fifty chemicals studied, only 1 out of 5 exhibit bioaccumulation factors on the order of 10X or less; while many exhibit bioaccumulation factors >100X. It is also interesting to note that the decrease in 14C-residues observed in catfish and minnows, despite continued exposure, suggests some type of enzyme induction process may occur. Similar phenomena have been previously reported (Macek et al., 1975).

(E) Toxicity To Critical Life Stages Studies - During the critical life stage studies with both fathead minnows and channel catfish the water hardness ranged from 26-34 mg/l $CaCO_3$, dissolved oxygen ranged from 60-95% saturation. The percent hatch of channel catfish eggs was unaffected by exposure to concentrations of nitroglycerine as high as 1.25 mg/l (Table 11). After 11 days exposure of fry, and during the remainder of the exposure period, we observed that catfish fry exposed to 1.25 and 0.62 mg/l nitroglycerine were lethargic and tended to remain stationary on the bottom of the test chamber. When these fish attempted to swim, they lost equilibrium and moved erratically about the test chamber. Exposure for 30 days to 1.25, 0.62, and 0.31 mg/l nitroglycerin significantly reduced

survival of catfish fry when compared to either the control or solvent control (Table 11), although no mortality was observed during the first 11 days of exposure. Exposure for 30 days to 1.25 and 0.62 mg/l nitroglycerine significantly reduced total length of surviving channel catfish fry when compared to controls (Table 11). No significant effects of exposure of catfish fry to 0.15 mg/l nitroglycerine were observed.

During the first study, continuous exposure of fathead minnow eggs to 1.87 and 0.94 mg/l nitroglycerine significantly reduced the percent hatch when compared to either the control or solvent control (Table 12). Exposure of fathead minnow fry to these two concentrations also resulted in complete mortality of the fry within 7 days, and exposure to 0.47 and 0.23 mg/l resulted in complete mortality of fry in 14 days. Percentage survival of fry exposed to 0.12 mg/l nitroglycerine for 30 days was significantly less than controls. Total length of surviving fathead minnow fry was unaffected by exposure to nitroglycerine.

In an effort to verify the results of the first fathead minnow study, a second study was initiated. Percentage hatch was significantly reduced by exposure to 3.75 mg/l when compared to the controls, while percentage survival at 7 days post hatch was significantly reduced at all treatment levels (Table 13). The test was terminated at this time.

in the test water by means of a rocker-arm apparatus driven by

A third study was then initiated with concentrations ranging from 0.47 to 0.03 mg/l. Hatchability of eggs was not adversely effected by exposure to concentrations of nitroglycerine as high as 0.47 mg/l (Table 14). Due to a system malfunction on day 26 post hatch in the solvent control, precentage survival was significantly reduced. Therefore, these data were not used in the statistical analyses. Survival of fathead minnows at thirty days post hatch was significantly reduced by exposure to 0.47, 0.23, 0.12, and 0.06 mg/l. Mean total length of fry exposed to 0.23 mg/l was significantly less than the mean length of control fry while the mean total length of those fry exposed to 0.47 mg/l appeared uneffected. Due to the high mortality in these two treatments, these mean lengths may be considered suspect.

(F) Chronic Toxicity to Invertebrates - No empirical differences between controls and solvent controls for any of the parameters measured were observed for either daphnids or midge.

Continuous exposure of Daphnia magna to 25 mg/l nitroglycerine for 7 days significantly reduced survival when compared to controls (Table 15), and this reduction in survival was directly related to duration of exposure. Survival of daphnids exposed to 25 mg/l nitroglycerine for 21 days was reduced to only 4%, while control survival after 21 days was 78%. In addition, continuous exposure to 25 mg/l nitroglycerine for 21 days completely inhibited reproduction of daphnids. Exposure to 12.5

mg/l nitroglycerine through day 14 significantly reduced the numbers of young produced when compared to controls (Table 16). No significant effects of exposure to 6.2 mg/l nitroglycerine were observed during the first generation exposure of daphnids.

As observed during the first generation, no significant effects on survival were observed among daphnids exposed to concentrations of nitroglycerine as high as 12.5 mg/l (Table 15). Although there appears to be reduced survival among second generation daphnids exposed to 12.5 mg/l nitroglycerine variability between replicates precludes ascribing statistical significance to this observation. As was observed during the first generation, production of young by D. magna exposed to 12.5 mg/l nitroglycerine was significantly reduced when compared to controls. No significant effects of exposure of D. magna to 6.2 mg/l over two generations were observed. Production of young D. magna exposed for 35 days to 1.5 mg/l nitroglycerine was significantly lower than the controls. We do not believe this to be a nitroglycerine related effect since production of young by D. magna exposed to 3.1 and 6.2 mg/l was comparable to the controls.

Exposure to 25.0 and 12.5 mg/l nitroglycerine for 10 days significantly reduced survival of midge larvae (Table 17). Furthermore, emergence of adult midges was significantly reduced by exposure to 25.0 and 12.5 mg/l nitroglycerine. Finally, no egg masses were produced by adults which emerged from midge populations exposed to 25.0 or 12.5 mg/l nitroglycerine. Although survival was significantly lower at the exposure concentration of 1.5 mg/l,

exposure to 6.2 and 3.1 mg/l of nitroglycerine did not appear to adversely affect survival and emergence of midges. The absence of any toxicant related trends at these exposure levels suggests that this reduction in survival is not nitroglycerine related. Since no egg masses were produced by the first generation midges, no observation of the effects of exposure of second generation midges to 25.0 or 12.5 mg/l nitroglycerine was possible. Survival of F_1 larvae exposed to 6.2 and 3.1 mg/l was significantly reduced. As was observed during the first generation exposure, pupae and adult survival, emergence of second generation midges, and production of egg masses was affected by continuous exposure to 1.5 mg/l nitroglycerine (Table 18).

(G) Chronic Toxicity to Fathead Minnows - The results of the analysis of water quality parameters measured throughout the chronic exposure indicated that hardness, alkalinity, acidity, dissolved oxygen and pH varied minimally between exposure aquaria during the test (Table 19).

Percentage survival and total lengths of fathead minnow fry were not affected by 30 days exposure to concentrations of nitroglycerine as high as 1.75 mg/l (Table 20). After 60 days, survival of fathead minnows was significantly reduced among fish exposed to 1.75, 0.87, 0.43 and 0.22 mg/l of nitroglycerine when compared with controls. Total lengths of fish exposed to concentrations as high as 1.75 mg/l for 60 days were not significantly different from those of controls. Between days 60 and 174 of exposure, none of the 15 fish selected for continued exposure to 1.75 mg/l of nitroglycerine had survived and

only 2 fish survived exposure to 0.87 mg/l nitroglycerine during this period. Survival through 174 days of exposure was also significantly reduced among fish exposed to 0.43 mg/l when compared with controls and lower concentrations of nitroglycerine.

At the time adult fish were terminated (day 266), wet weight of male fish exposed to 0.43 mg/l was significantly less than that of male fish in controls and lower concentrations of nitroglycerine (Table 21). When examined, the male fish in the A duplicate of 0.43 mg/l was sexually immature, a fact which accounts for the lack of spawning in this tank.

Lengths and weights of female fathead minnows were not effected by 266 days exposure to concentrations of nitroglycerine as high as 0.43 mg/l (Table 21). The total spawns, total eggs, spawns per female and eggs per female appeared reduced among fish exposed to 0.43 and 0.22 mg/l of nitroglycerine. However, the variability in these parameters between duplicates of solvent control and fish exposed to 0.11 mg/l precluded ascribing statistical significance to these observations.

Hatchability of eggs from one spawn which occurred among fish exposed to 0.43 mg/l was reduced when compared with hatchability of eggs spawned in controls and lower concentrations of nitroglycerine (Table 22). Hatchability of control eggs incubated in 0.43 mg/l nitroglycerine was similar to hatchability of eggs incubated in the control. With the exception of one egg group incubated in the A duplicate of 0.87 mg/l of nitroglycerine,

hatchability of control eggs incubated in 1.75 and 0.87 mg/l of nitroglycerine was virtually 0%.

Survival after 30 days exposure of control fry transferred to 1.75, 0.87, and 0.43 mg/l nitroglycerine was similar to survival of fry reared in control and solvent control treatments. However, total length and wet weight of fry transferred to these same concentrations was lower than that of controls. Survival of fry from eggs spawned by fish exposed to 0.22 mg/l of nitroglycerine and reared for 30 days was reduced when compared with controls and second generation fry exposed to 0.11 mg/l. Total length and wet weight of fry exposed to 0.22 mg/l was significantly reduced when compared with controls and second generation fry exposed to 0.11 mg/l.

DISCUSSION AND CRITERIA FORMULATION

The data from all of the critical life stage studies with fathead minnow appear to be at variance with the data concerning the exposure of the critical life stages during the first generation of the fathead minnow chronic study, but appear to coincide with the data concerning the exposure of critical life stages during the second generation of the fathead minnow chronic study. This discrepancy in the data can be explained by the fact that the egg and fry study was initiated with newly fertilized eggs (<24 hour old) while the chronic study was initiated with newly hatched fry (< 24 hour old). According to the studies on the acute toxicity of nitroglycerine to selected life stages of the fathead minnow, the egg stage was determined to be the most sensitive life stage, and the newly hatched fry exhibited the greatest tolerance. Additionally, eggs of the second generation fathead minnows were significantly affected from the high concentration of 1.75 mg/l to a concentration of 0.43 mg/l, which agrees substantially with the information gathered during the egg and fry studies. This appears to indicate one of the mechanisms of the toxicity of nitroglycerine is through increased permeability of the chorion of the egg. Although a relatively high concentration of acetone was present during the first critical life stage study of the fathead minnow, the data from the solvent control indicate little or no effect of acetone on any life stages tested. Furthermore, substantially

identical results were observed during exposure of the second and third critical life stage studies and in the F_1 control eggs to similar concentrations of nitroglycerine despite the fact that acetone concentrations were significantly less.

The generally accepted expression for that concentration of a water quality constituent reasonably judged to preclude hazard to aquatic organisms due to the presence of that constituent is the development of a water quality criterion.

In general, water quality criteria specify concentrations of water constituents which when not exceeded will protect one organism, an organism community, or a prescribed water quality use with an adequate margin of safety during continuous chronic exposure. The procedures for genesis of valid water quality criteria have been well defined (National Technical Advisory) Committee, 1968; National Academy of Sciences, National Academy of Engineering, 1972; U.S. Environmental Protection Agency, 1971c). Water quality criteria reflect a knowledge of both environmental accumulation and persistence, and acute and chronic toxicity of specific toxicants in aquatic ecosystems. The most critical aspect in formulating a valid water quality criterion is developing an understanding of the relationship between the acute and chronic toxicity of a chemical to aquatic organisms. Mount and Stephan (1967) have suggested this relationship is constant for closely related species (i.e. fishes); and Macek et al. (1976) have provided data to suggest that the

relationship may be constant across broad taxonomic groups of aquatic organisms (i.e. fishes, aquatic invertebrates) and therefore can be utilized to estimate chronically safe levels for species based on acute toxicity data.

Based on the broad spectrum of acute toxicity data, information of the accumulation of nitroglycerine residues in biological systems, a clear understanding of the relationship between the acute and chronic toxicity of nitroglycerine to aquatic organisms and a reasonable data base which supports the applicability of this relationship to broad taxonomic groups, we believe these research efforts provide a sound data base for the formulation of a valid water quality critia for nitroglycerine for the protection of freshwater aquatic life with an adequate margin of safety.

Results of acute toxicity tests, both static and flow-through, with a wide variety of freshwater aquatic organisms representing several trophic forms under a variety of water quality conditions indicate that the acute LC50 values generally are greater than 0.5 mg/l nitroglycerine (Tables 1, 4, 5, 6 and 9). The data from the bioconcentration study utilizing 14C-nitroglycerine and several species of fish clearly indicate that nitroglycerine has a relatively low bioconcentration potential (Table 10). Thus, the critical aspect of a criterion formulation is accurately defining the specific empirically-derived application factor for nitroglycerine which should be utilized to estimate the chronic toxicity of this chemical to aquatic organisms based

on available acute toxicity data.

We have determined during flow-through acute toxicity tests that the 96-hour LC50 of nitroglycerine to fathead minnows is 3.0 mg/l. We have further determined during chronic toxicity studies with fathead minnows that the chronically safe concentrations (MATC) based on toxic effects over one complete life cycle is between 0.11 and 0.22 mg/l. Thus based on an understanding of the chronic toxicity of nitroglycerine to fathead minnows, the limits of the empirically defined ratio (application factor) specific for nitroglycerine are >0.11/3.0 and <0.22/3.0, or >0.037<0.073. In addition, in a separate embryo-larval study with fathead minnows we have determined the safe concentration to be between 0.03 and 0.06 mg/l, and estimated the application factor to be >0.01<0.02. We have determined the 96-hour LC50 of nitroglycerine to channel catfish during a static bioassay to be 4.7 mg/l. Based on an embryo-larval exposure we have estimated the MATC for nitroglycerine and channel catfish to be >0.15<0.31 mg/l, and estimated the application factor for nitroglycerine based on larval or channel catfish to be between >0.032<0.066. We have further tested the applicability of the application factor to other aquatic organisms by empirically investigating the chronic toxicity of nitroglycerine to daphnids and midges. We have determined during flow-through acute toxicity tests that the 48-hour LC50 of nitroglycerine to daphnids and midges is 32 mg/l and 20 mg/l, respectively. During chronic exposure with these species, we have further determined that the chronically safe concentrations of nitroglycerine for daphnids and midges

are estimated to be >6.2<12.5 mg/l. Thus the limits on the empirically defined ratio (application factor) specific for nitroglycerine are >6.2/32<12.5/32, or >0.2<0.4 based on daphnids, and are >6.2/20<12.5/20, or >0.3<0.6 based on midges. Clearly, the estimates for the application factor specific for nitroglycerine based on these diverse species of aquatic organisms suggest that the application factor for fish can be conservatively utilized with confidence to generate a valid water quality criteria for nitroglycerine.

Based on a conservative analysis we have applied the lower limit of all estimates of the application factor specific for nitroglycerine (0.01) to the lower limit of the observed acute toxicity values for the appropriate freshwater organisms (1.38 mg/l, 96-hour LC50 for bluegill at pH 6.0). As a result of this analysis, we propose that a concentration of 0.01 mg/l nitroglycerine would be a reasonable water quality criterion providing for the protection of freshwater life with an ample margin of safety.

CONCLUSIONS

1. The estimated LC50 values for a wide variety of freshwater organisms are generally greater than 1.0 mg/l.

2. Nitroglycerine was generally more toxic to fishes than to invertebrates or algae. Additionally, it was generally more toxic to invertebrates than to algae.

3. Results of dynamic testing with fish and invertebrates suggest cumulative toxicity of nitroglycerine as a result of duration of exposure is not significant.

4. Fish exposed to 14C-nitroglycerine exhibited an apparent lack of appreciable bioaccumulation (8-15X).

5. The limit on an application factor for critical life stages of the fathead minnow and channel catfish was determined to be >0.01<0.02 and >0.03<0.07, respectively.

6. The application factor for fathead minnows based on toxic effects over one complete life cycle is between >0.03 <0.07.

7. The limits on an application factor for daphnids and midges were determined to be >0.2<0.4 and >0.3<0.6, respectively.

8. A water quality criterion of 0.01 mg/l nitroglycerine is proposed for the protection of freshwater aquatic life.

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Table 1 -- Acute toxicity of nitroglycerine to the freshwater algae Selenastrum capricornutum, Navicula pelliculosa, Microcystis aeruginosa and Anabeana flos-aquae. The data are expressed as EC50 values (mg/ℓ) based on either decrease in number of cells/mℓ, optical density, or chlorophyll a concentration (based on nominal concentrations).

Species	EC50 (cells/mℓ) 24-hour	48-hour	96-hour	EC50 (chlorophyll a) 96-hour
S. capricornutum	4.0 (0.1-30.4)[a]	1.1 (0.1-22.8)	0.4 (0.1-1.3)	1.0 (0.7-7.0)
N. pelliculosa	>10.0	8.6 (1.2-59.9)	3.3 (0.3-33.0)	1.0
M. aeruginosa	>10.0	>10.0	>10.0	>10.0
A. flos-aquae (optical density)	>10.0	>10.0	>10.0	>10.0

[a] 95% confidence interval.

Table 2 -- Decrease in number of cells/mℓ or optical density as compared to controls[a] among algae exposed to nitroglycerine during 96-hour static toxicity tests (based on nominal concentrations).

Species	Nitroglycerine (mg/ℓ)	Decrease cells/mℓ (%) 24-hour	48-hour	96-hour
S. capricornutum	0.1	24	30	27
	0.3	24	35	25
	1.0	28	46	41
	1.8	31	45	66
	3.2	44	53	100
	10.0	73	84	100
N. pelliculosa	0.1	0	10	8
	0.3	2	8	9
	1.0	0	22	26
	1.8	8	21	38
	3.2	19	49	59
	10.0	13	53	68
M. aeruginosa	0.1	0	5	8
	0.3	5	3	15
	1.0	0	0	10
	1.8	5	4	7
	3.2	0	12	32
	10.0	0	6	27

Table 2 -- Continued.

Species	Nitroglycerine (mg/ℓ)	Decrease cells/mℓ (%) 24-hour	48-hour	96-hour
A. flos-aquae (optical density)	0.1	0	2	0
	0.3	0	0	0
	1.0	0[b]	0	0
	1.8	0	0	0
	3.2	0[b]	0	0[b]
	10.0	0	0	0[b]

[a] Control values represent unity against which all other measurements are compared. For S. capricornutum comparisons were made to the lactose treated control, for other species comparisons are made to untreated controls.

[b] Slight increase observed (1-3%).

Table 3 -- Decrease (%) in chlorophyll *a* concentrations of algae compared to control[a] cultures after 96-hours exposure to nitroglycerine (nominal concentrations).

Nitroglycerine (mg/ℓ)	*S. capricornutum*	*N. pelliculosa*	*M. aeruginosa*	*A. flos-aquae*
0.1	30	3	0	4
0.3	40	47	5	0
1.0	44	67	12	0
1.8	53	65	10	1
3.2	66	79	20	0
10.0	65	79	27	0

[a] Control values represent unity against which all measurements are compared.

chronic toxicity of this chemical to aquatic organisms based

Table 4 -- Toxicity of nitroglycerine to freshwater fishes and aquatic invertebrates during static acute toxicity tests (nominal concentration).

Species	24-hour	48-hour	96-hour
		LC50 (mg/ℓ)	
bluegill	6.4 (4.7-8.8)[a]	4.3 (2.7-6.8)	2.7 (2.0-3.7)
rainbow trout	4.8 (3.6-6.6)	2.9 (2.1-3.9)	2.8 (2.0-3.8)
channel catfish	6.5 (4.8-8.8)	4.7 (3.5-6.4)	3.2 (2.6-3.8)
fathead minnow	6.3 (4.6-8.6)	4.1 (3.4-5.0)	2.5 (2.0-3.0)
		EC50 (mg/ℓ)	
water flea	51 (35-72)	46 (38-55)	-[b]
scud	74 (55-99)	50 (41-60)	-
sowbug	57 (46-72)	50 (38-66)	-
midge	76 (57-102)	55 (47-64)	-

[a] 95% confidence interval.

[b] Invertebrate tests were 48 hours in duration.

safe concentrations of nitroglycerine for daphnids and midges

Table 5 -- Acute toxicity of nitroglycerine to selected life stages of the fathead minnow (Pimephales promelas) (nominal concentration).

Life stage	LC50 (mg/ℓ) 24-hour	48-hour	96-hour	144-hour
eggs	>18.0	>18.0	>18.0	1.2 (0.7-2.1)[a]
1-hour fry	>10.0	>8.7<10.0	5.5 (4.6-6.6)	-[b]
7-day fry	5.4 (4.5-6.6)	3.4 (3.0-3.9)	2.1 (1.7-2.6)	-
30-day fry	6.8 (5.0-9.2)	4.8 (3.9-5.8)	2.1 (1.6-2.6)	-
60-day fry	5.3 (4.3-6.4)	4.1 (3.4-5.0)	3.4 (2.8-4.2)	-

[a] 95% confidence interval.

[b] Tests with fry were 96 hours in duration.

Table 6 -- Acute toxicity of nitroglycerine to bluegill (Lepomis macrochirus) under varying conditions of water quality[a] (nominal concentration).

Water quality	LC50 (mg/ℓ) 24-hour	48-hour	96-hour
15°C	6.38 (4.01-10.20)	6.09 (3.80-9.76)	3.55 (1.92-6.59)
20°C	6.23 (3.90-9.94)	4.82 (3.45-6.73)	1.92 (0.88-4.16)
25°C	6.67 (4.18-10.60)	4.91 (3.41-7.07)	1.99 (1.28-3.09)
35 mg/ℓ hardness	6.67 (4.18-10.60)	2.72 (1.79-4.13)	1.76 (1.12-2.74)
100 mg/ℓ hardness	4.01 (2.11-7.59)	2.71 (1.79-4.13)	1.51 (0.99-2.32)
250 mg/ℓ hardness	6.18 (3.87-9.87)	3.90 (2.08-7.34)	1.65 (1.07-2.55)
pH 6.0	6.18 (3.87-9.87)	3.90 (2.08-7.34)	1.38 (0.87-2.21)
pH 7.0	5.04 (3.53-7.21)	3.10 (1.89-4.97)	1.91 (0.88-4.14)
pH 8.0	6.39 (4.01-10.20)	6.09 (3.80-9.76)	2.10 (0.97-4.54)

[a]Under varied conditions of temperature (15, 20, 25°C) the pH was maintained at 7.1, and the hardness was 35 mg/ℓ. Under varied conditions of hardness (35 mg/ℓ, 100 mg/ℓ, 250 mg/ℓ) the pH was maintained at 7.1, and the temperature was 20°C. Under varied conditions of pH (6.0, 7.0, 8.0), hardness was maintained at 35 mg/ℓ, and the temperature was 20°C.

respectively.

Table 7 -- Acute toxicity of "aged" nitroglycerine solution to bluegill (Lepomis macrochirus) (nominal concentration).

Age of solution (hrs.)	LC50 (mg/ℓ) 24-hour	48-hour	96-hour
0	6.1 (3.8-9.8)[a]	3.8 (2.0-7.0)	2.0 (0.9-4.4)
12	4.6 (3.3-6.5)	3.7 (2.0-6.8)	2.0 (0.9-4.4)
24	6.2 (3.8-9.7)	2.7 (1.8-4.1)	2.0 (0.9-4.3)
48	3.9 (2.6-5.8)	2.2 (1.5-3.4)	1.7 (1.1-2.7)
96	4.7 (3.4-6.6)	3.6 (2.0-6.7)	2.1 (1.0-4.6)

[a] 95% confidence interval.

Table 8 -- Change with time in the relative concentrations of nitroglycerine during static bioassays under various water quality conditions.

Water quality	hour/	% of nitroglycerine added remaining in solution			
	0	24	48	72	96
Standard conditions	100	87	82	85	74
15°C	100	105	93	90	81
25°C	100	90	95	87	82
100 mg/ℓ $CaCO_3$	100	92	86	80	82
250 mg/ℓ $CaCO_3$	100	83	80	82	71
pH 6.0	100	100	90	84	85
pH 8.0	100	79	83	81	75

[a] Standard conditions were 20°C, 35 mg/ℓ hardness, and pH 7.1. Where one parameter was varied from standard, the others were maintained at standard.

Table 9 -- Toxicity of nitroglycerine to freshwater fishes and aquatic invertebrates during flow-through acute toxicity tests.

Species	LC50 (mg/1) 24-hour	48 or 96-hour[a]	Incipient[b]
bluegill	> 1.87	1.67 (0.87-3.25)	0.55 (0.45-0.70)
channel catfish	> 1.87	>1.87	0.50 (0.37-0.65)
fathead minnow	> 6.0<8.0	3.0 (2.2-3.7)	1.87 (1.32-2.75)
water flea	72 (15-350)	32 (21-50)	25 (12-52)
midge	57 (18-182)	20 (11-37)	18 (10-32)

[a] 48-hour LC50 for water flea and midge, 96-hour LC50 for fishes.

[b] Incipient LC50 for water flea and midge estimated after 96 hours exposure; incipient LC50 estimated after 384 hours for bluegill, 435 hours for catfish and 288 hours for fathead minnow.

[c] 95% confidence interval.

Table 10 -- Mean[a] measured concentrations of 14C-residues in the edible portion of fishes during 8 days continuous exposure to 14C-nitroglycerine in flowing water.

Day	14C-nitroglycerine (mg/l)	14C-residues (mg/kg) in tissues: bluegill	rainbow trout	channel catfish	fathead minnow
1	0.37 (0.05)[b]	2.7 (0.35)	3.0 (0.24)	2.0 (0.7)	1.9 (0.17)
2	0.42 (0.05)	3.7 (0.3)	2.5 (0.17)	2.5 (0.5)	3.2 (0.3)
4	0.55 (0.10)	5.7 (0.3)	3.5 (0.81)	3.0 (1.0)	3.0 (0.3)
8	0.32 (0.10)	6.2 (0.88)	3.3 (0.2)	1.0 (0.1)	1.8 (0.6)
Mean	0.42 (0.10)	-	-	-	-

[a]Mean measured 14C-nitroglycerine concentrations based on radiometric analysis of triplicate samples; mean measured 14C-residues in fish based on analysis of duplicate portions of each of four fish, N=8.

[b]Standard deviation.

Table 11 -- Mean percentage hatch of eggs and survival and mean total length of channel catfish (Ictalurus punctatus) fry continuously exposed to replicate concentrations of nitroglycerine in flowing water for 30 days.

Nominal concentration (mg/l)	Replicate	Hatch (%)	Survival (%)	Length (mm)
1.25	A	82	26[a]	18 ± 2[b]
	B	63	30	18 ± 1
0.62	A	100	70[a]	21 ± 2[b]
	B	92	74	21 ± 2
0.31	A	79	72[a]	23 ± 2
	B	74	88	23 ± 2
0.15	A	84	84	23 ± 3
	B	85	86	23 ± 2
0.08	A	86	90	24 ± 3
	B	85	94	24 ± 2
control	A	90	96	23 ± 2
	B	72	92	24 ± 3
solvent control	A	86	92	24 ± 2
	B	91	90	23 ± 2

[a] $F=32.68$ $F_{0.05}(6,6) = 4.28$; $d'=10.95$

[b] $F=53.87$ $F_{0.05}(6,6) = 4.28$; $d'=1.19$

Table 12 -- Mean percentage hatch of eggs, and survival and mean total length of fathead minnow (Pimephales promelas) fry continuously exposed to replicate concentrations of nitroglycerine in water for 30 days during the first experimental exposure.

Nominal concentration (mg/l)	Replicate	Hatch (%)	Survival (%)	Length (mm)
1.87	A	9[a]	0	-
	B	7	0	-
0.94	A	24[a]	0	-
	B	44	0	-
0.47	A	64	0	-
	B	86	0	-
0.23	A	92	0	-
	B	86	0	-
0.12	A	86	32[b]	15 ± 4
	B	96	48	16 ± 2
control	A	95	70	18 ± 3
	B	95	90	17 ± 2
solvent control	A	94	74	16 ± 3
	B	88	62	15 ± 3

[a] $F=26.7$ $F_{0.05}(6,6) = 4.28$; $d'=18.63$

[b] $F=54.32$ $F_{0.05}(6,6) = 4.28$; $d'=15.26$

Table 13 -- Mean percentage hatch of eggs and survival of fathead minnow (Pimephales promelas) fry continuously exposed to replicate concentrations of nitroglycerine in water for 7 days.

Nominal Concentration (mg/l)	Replicate	Hatch (%)	Survival 7 days post hatch (%)
3.75	A	50[a]	0[b]
	B	54	0
1.87	A	94	4[b]
	B	89	4
0.94	A	93	26[b]
	B	89	22
0.47	A	96	24[b]
	B	86	4
0.23	A	97	4[b]
	B	94	10
control	A	97	86
	B	99	74
solvent control	A	89	84
	B	96	92

[a] $F=15.5$ $F_{0.05}(5,5) = 4.28$; $d'=12.26$

[b] $F=38.148$ $F_{0.05}(6,6) = 4.28$; $d'=17.82$

Table 14 -- Mean percentage hatch of eggs, and survival and mean total length of fathead minnow (Pimephales promelas) fry continuously exposed to replicate concentrations of nitroglycerine in water for 30 days.

Nominal concentration (mg/l)	Mean measured concentration[a] (mg/l)	Replicate (%)	Hatch (%)	Survival (%)	Total length (mm)
0.47	0.48	A	70	6[c]	23 ± 1
		B	87	0	-
0.23	0.25	A	86	10[c]	19 ± 3[d]
		B	94	0	-
0.12	0.13	A	90	62[c]	24 ± 4
		B	96	48	22 ± 4
0.06	0.06	A	94	84[c]	24 ± 3
		B	93	74	25 ± 1
0.03	0.03	A	90	94	23 ± 4
		B	93	84	24 ± 3
control	-	A	94	90	25 ± 2
		B	90	88	25 ± 2
solvent control	-	A	97	18[b]	25 ± 2
		B	94	12	26 ± 2

[a] Analysis performed by the U. S. Army Medical Research & Development Command.

[b] Low survival in the solvent control was due to a system malfunction occurring on day 26 post hatch.

[c] $F=39.15$ $F0.05\ (5,5) = 5.05$; $d'=9.21$

[d] $F=15.0$ $F0.05\ (5,5) = 5.05$; $d'=2.39$

Table 15 -- Mean (standard deviation) percentage survival of Daphnia magna exposed to nitroglycerine for two generations. Each value represents the mean of four replicate treatments.

Nominal concentration (mg/ℓ)	Average percent survival					
	Generation I			Generation II		
Day/	7	14	21	28	35	42
controls	96(8)	88(6)	78(6)	98(5)	96(8)	94(8)
1.5	85(7)	80(7)	78(6)	91(8)	90(11)	89(9)
3.1	89(8)	89(8)	88(6)	86(9)	86(9)	84(11)
6.2	91(8)	89(8)	82(13)	91(8)	89(13)	88(12)
12.5	90(4)	80(11)	76(14)	68(35)	68(35)	65(32)
25.0	58(30)[a]	26(13)[b]	4(8)[c]	–[d]	–	–

[a] F=3.86 F0.05 (5,15) = 2.90; d'=19.9

[b] F-11.07 F0.05 (5,15) = 2.90; d'=16.7

[c] F=29.93 F0.05 (5 15) = 2.90; d'=15.4

[d] No young produced in 1st generation to initiate 2nd generation.

Table 16 -- Mean (standard deviation) young produced per parthenogenetic female Daphnia magna exposed to nitroglycerine for two generations. Each value represents the mean of four replicates (nominal concentration).

Nitroglycerine (mg/ℓ)	Young produced/parthenogenetic female			
	Generation I		Generation II	
	14	21	35	42
controls	6(2)	10(3)	10(4)	14(6)
1.5	9(6)	14(6)	4(1)	16(6)
3.1	7(2)	15(5)	7(5)	14(4)
6.2	5(2)	10(4)	5(1)	15(2)
12.5	1(2)[a]	7(4)	1(1)[c]	9(2)
25.0	0(0)[a]	0(0)[b]	-[d]	-

[a] $F=5.97$ $F_{0.05}(5,15) = 2.90$; d'=4.9

[b] $F= 2.90$ $F_{0.05}(5,15) = 2.90$; d'=9.2

[c] $F=4.58$ $F_{0.05}(4,12) = 3.26$; d'=5.4

[d] No young produced in 1st generation to initiate 2nd generation.

Table 17 - Mean[a] (S.D.) percentage survival of first generation[b] (Chironomus tentans) larvae, pupae, adults, and percent emergence of adults during continuous exposure to nitroglycerine (nominal concentration).

Nitroglycerine (mg/l)	larvae	pupae	adults	Emergence (%)	Eggs/Adult
Controls	69(18)[c]	95(5)	98(1)	100(0)	9
1.5	48(7)[d]	83(24)	97(6)	85(27)	6
3.1	69(8)	92(15)	91(16)	92(16)	17
6.2	53(16)	87(12)	77(34)	80(23)	6
12.5	36(16)[d]	64(26)	62(12)[e]	88(18)	0
25.0	28(31)[d]	83(15)	16(31)[e]	43(42)[f]	0

[a] Mean based on four replicates.

[b] Survival of larvae determined after 10 days exposure, all other measurements recorded after 18 days exposure.

[c] Standard deviation.

[d] $F=4.27$ $F_{0.05}(5,15) = 2.90$; $d'=17.8$

[e] $F=6.29$ $F_{0.05}(5,15) = 2.90$; $d'=31$

[f] $F=3.36$ $F_{0.05}(5,15) = 2.90$; $d'=29.1$

Table 18 - Mean[a] (S.D.) percentage survival of second generation Chironomus tentans larvae, pupae, adults, and percent emergence of adults to nitroglycerine (nominal concentration)

Nitroglycerine (mg/l)	Survival %			Emergence (%)	Eggs/Adult
	larvae	pupae	adults		
Controls	58(9)[c]	100(1)	74(19)	71(29)	5
1.5	61(11)	98(2)	52(25)	94(9)	21
3.1	33(7)[d]	83(24)	80(17)	75(49)	6
6.2	48(7)[d]	96(4)	66(14)	100(0)	18

[a]Mean based on four replicates.

[b]Survival of larvae determined after 13 days exposure, all other measurements recorded after 27 days exposure.

[c]Standard deviation.

[d]F=9.86 F0.05 (3,9) = 3.86; d'=7.9

Table 19 - Measured water quality parameters during chronic exposure of fathead minnows (Pimephales promelas) to nitroglycerine.

Parameter	Number of samples	Mean and Standard deviation	Range
Hardness (mg/l)	14	40 $\pm$ 2	38-42
Alkalinity (mg/l)	5	46 $\pm$ 6	42-53
Acidity (mg/l)	6	4.6 $\pm$ 1.1	3.8-6.7
Dissolved oxygen (mg/l)	469	7.6 $\pm$ 0.6	5.5-8.9
pH	63	-	6.9-7.6

Table 20 -- Survival and growth of fathead minnow (Pimephales promelas) continuously exposed to nitroglycerine. (nominal concentration).

Parameter	Nitroglycerine (mg/l) 1.75 A	1.75 B	0.87 A	0.87 B	0.43 A	0.43 B	0.22 A	0.22 B	0.11 A	0.11 B	Solvent[a] A	Solvent[a] B	Control A	Control B
30 Days														
survival (%)	98	100	98	75	85	83	93	93	100	100	98	98	100	98
total length (mm)	18(2)[b]	17(3)	17(3)	17(3)	16(4)	16(4)	16(3)	18(4)	17(3)	18(3)	18(2)	18(3)	18(2)	17(3)
60 Days														
survival (%)	78[d]	85	58[d]	58	78[d]	73	85[d]	83	100	90	98	93	100	98
total length (mm)	28(4)	26(4)	27(4)	26(6)	25(5)	24(6)	26(4)	25(6)	26(5)	25(4)	27(3)	26(4)	26(4)	25(4)
174 Days														
survival (%)[c]	0[e]	0	13[e]	0	60[e]	80	93	87	93	93	100	100	100	100

[a] Solvent control.

[b] Mean and standard deviation.

[c] Based on 15 fish per duplicate (after thinning on day 60).

[d] $F=7.29$ $F_{0.05}(6,6) = 4.28$; $d'=19.0$

[e] $F=55.26$ $F_{0.05}(6,6) = 4.28$; $d'=20.44$

Table 21-- Size and reproduction potential of fathead minnows continuously exposed to nitroglycerine for 266 days (nominal concentration)

Parameter	Nitroglycerine (mg/l) 1.75 A	1.75 B	0.87 A	0.87 B	0.43 A	0.43 B	0.22 A	0.22 B	0.11 A	0.11 B	Solvent[a] A	Solvent[a] B	Control A	Control B
266 Days														
Total Length (mm)														
males	-	-	-	-	60	57	64	66	63	65	74	66	65	63
females	-	-	-	-	53	53	54	53	53	49	55	55	53	52
Wet Weight (g)														
males	-	-	-	-	1.96[b]	2.14	3.62	3.60	3.97	3.78	4.66	3.60	3.26	3.29
females	-	-	-	-	1.48	1.69	2.03	1.63	1.66	1.21	1.63	1.80	1.47	1.37
Males/female	-	-	-	-	1/5	3/5	2/6	3/5	3/6	3/6	3/6	3/6	4/3	3/6
Total spawns	-	-	-	-	0	1	3	6	3	16	3	23	20	27
Total eggs	-	-	-	-	0	131	190	493	163	1166	457	2105	1738	2067
Spawns/female	-	-	-	-	0	0.2	0.5	1.0	0.5	2.7	0.5	3.8	6.6	4.5
Eggs/female	-	-	-	-	0	26	31	82	33	194	76	351	579	345
Eggs/spawn	-	-	-	-	0	131	63	82	54	73	152	73	87	77

[a] Solvent control.

[b] F=10.9 F0.05 (4,4)=6.39; lsd (0.05)=0.97.

Table 22-- Hatchability of eggs, and survival and growth of second generation fathead minnow fry continuously exposed to nitroglycerine (nominal concentration).

Parameter	Nitroglycerine (mg/l)													
	1.75		0.87		0.43		0.22		0.11		Solvent[a]		Control	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Hatchability (%)														
Eggs from parents (N)[b]	-	0	-	-	-	58(1)	82(2)	93(3)	88(1)	92(5)	95(3)	89(9)	97(8)	94(13)
Eggs transferred from control (N)	2(3)	0(3)	96(1)	3(3)	96(2)	84(1)	-	-	-	-	-	-	-	-
30 Days														
Survival (%) (N)[c]	90(2)[e]	88(2)[e]	70(2)	90(2)[e]	73(2)[e]	53(2)	18(2)	38(2)	80(1)	58(3)	100(2)	81(5)	71(4)	90(4)
Total length (mm)	17(3)[f]	16(4)	22(2)[f]	18(3)	19(4)[f]	18(4)	18(6)[f,g]	18(6)	22(4)	22(5)	22(2)	21(5)	22(4)	21(4)
Mean wet weight (g)	0.05[h]	0.06	0.09[h]	0.05	0.07[h]	0.05	0.06[h,i]	0.06	0.10	0.12	0.10	0.10	0.10	0.09

[a] Solvent control.

[b] Number of egg groups exposed.

[c] Mean and standard deviation.

[d] Number of fry groups exposed.

[e] Fry transferred from control after hatch.

[f] Including transferred groups-F=9.74 F0.05(6,6)=4.28;d'=2.77

[g] Excluding transferred groups-F=41.02 F0.05(3,3)=9.28; lsd(0.05)=1.31

[h] Including transferred groups-F=5.18 F0.05 (6,6)=4.28; d'=4.12

[i] Excluding transferred groups-F=11.95 F0.05 (3,3)=9.28; lsd(0.05=0.03

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